ARTIFICIAL SPAWNING OF CARP IN A SUBTROPICAL CLIMATE CONDITIONS

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VEŠTAČKI MREST ŠARANA U USLOVIMA SUBTROPSKE KLIME

Abstrakt

Najefikasniji i najpouzdaniji način proizvodnje ikre i ribljeg podmlatka je veštački mrest. Matične jedinke se drže u vodi koja je zasićena sa kiseonikom i čija je temperatura u rasponu 20-24 ºC. Daju im se dve doze injekcije hormona hipofize ili mešavina GnRH/dopamin antagonista da bi se indukovala ovulacija i spermatogeneza. Otkriće da dopamin deluje kao inhibitorni faktor za sintezu gonadotropina dovelo je do revolucije u razvoju veštačkog mresta. Od sredine osamdesetih, hipofizacija je unapređena uvođenjem standardizovanog suvog ekstrakta hipofize šarana u kojem je sadržaj i aktivnost luteinizirajućeg hormona (LH) kalibrisana (cCPE). U suptropskim klimatskim uslovima, šaran sazreva u starosti od šest meseic, a sezona mresta šarana počinje krajem februara i početkom marta, kada temperatura vode dostigne 19-21°C.

Ogled je izveden u Gan Shmuel mrestilištu i centru za gajenje riba, u Izraelu, u decembru. Trideset ženki KOI šarana (Cyprinus carpio L.) bilo je smešteno u deset plastičnih bazena, zapremine oko 450 litara. U svakom tanku je bilo po tri matice. Mužjaci su držani odvojeno od matica da bi se sprečila nekontrolisana reprodukcija. U svakom bazenu aeracija i stalno snabdevanje svežom vodom su bili kontrolisani. Pošto je veštački mrest sproveden van sezone, 21 dan pre izvođenja mresta ribe su držane u vodi na temperaturi od 24°C. Urađena je biopsija po jedne ženke iz svake grupe. Anestezija ženki je izvršena sa 99% 2 phenoxyetanolom i biopsija jajnika je urađena preko genitalnog otvora, uvođenjem plastičnog katetera do gonada. Procenat oocista koje su bile u stadijumu I (centralno postavljen GV) i procenat oocista u stadijumu II (ekscentričan GV) je određen posmatranjem pod binokularnim mikroskopom. Pozicija germinalnog vezikula i zrelost matica je određena i broj oocisti u svakon stadijumu je izračunat. Ukupna telesna masa svih matica je izračunata i iznosila je oko 40 kg. cCPE koncentar-

cije 1 ml/kg je pripremljen za sve jedinke zajedno. Hipofizacija riba je izvršena u dva koraka. Primarna doza je iznosila 20% od ukupne doze, dok je druga doza iznosila 80% od ukupne doze. Deset mužjaka je dobilo po jednu dozu cCPE, 70% doze od one koja je data maticama. Sledećeg dana, došlo je do mrešćenja nešto kasnije, nego što je očekivano. Ovo se može objasniti time što je ogled sproveden izvan sezone mresta. Period latence je takođe visoko zavisan od temparature vode. Tokom ogleda došlo je do malog problema sa sistemom za grejanje, pa je to još jedan od razloga za nešto produžen period latence. Jaja su nežno istisnuta u suvu posudu i oplođena primenom "suve metode", a adhezivnost jaja je eliminisana korišćenjem mleka ili tretiranjem sa solima uree, a zatim sa kupkom u taninskoj kiselini ("Woynarovich method"). Inkubacija je sprovedena u Cuger bocama.

Za statističku obradu podataka korišćen je SPSS za Windows i Excel (MS Office). Uspešnost mresta je iznosila 43 %. Izračunat je Spearmanov koeficijent korelacije između mase ženki i mase dobijene ikre, korelacija između mase riba i broja jaja i korelacija između procenta migriranih oocita i uspešnosti mresta. Uočena je značajna korelacija (F=0,709) između telesne mase matica i mase ikre i značajna korelacija (F=0,642) između telesne mase matica i broja jaja. Negativna korelacija (F=-0,530) između procenta migriranih oocista i uspešnosti mresta je u suprotnosti sa mnogim ranijim istraživanjima. Petpostavka je da bi razlog ovome mogao biti u faktorima okoline, kao i u kvalitetu samih matica. Iako je procenat izmrešćenih matica bio niži od normalnog procenta uspešnosti veštačkog mresta šarana, uspešnost je bila vrlo zadovoljavajuća imajući u vidu da je mrest izvršen van sezone mresta.

Ključne reči: *veštački mrest, KOI šaran, cCPE, vansezonski mrest, suptropska klima*

INTRODUCTION

Artificial spawning is the most effective and the most reliable method of eggs and fingerlings production and control of infectious and parasitic diseases. Controlled spawning began around year 1725th when L. Jacobi succeeded to control fertilization of eggs of salmon and trout (Hristić and Bunjevac, 1991; Treer et al, 1995). The major breakthrough in fish breeding came in with the finding that dopamine acts as inhibitory factor for synthesis of gonadotropin (Peter et al, 1986). This breakthrough led to the development of the artificial spawning. Common carp matures in subtropical climate zone at six months (Sarig, 1966). An adult common carp may spawn four or five times per year in subtropical conditions if maintained at 20 to 22°C (Horvath, 1986). Diversity in fish reproductive strategy involves diversity in the timing of fish spawning (Webb and McLay, 1996). Thus, it occurs in late spring to summer in carp in Europe (Billard, 1995). In Israel the spawning seasons starts with common carp at the end of February and beginning of March, when the water temperatures reach 19-21°C. (Jovanović and Ristić, 1960). Since the middle 1980s, hypophysation has improved through the introduction of a standardized dry carp pituitary extract in which the luteinizing hormone (LH) content and activity have been calibrated (calibrated carp pituitary extract = cCPE) (Yaron et al, 2009). Approximately 300 000 to 800 000 newly hatched fry can be expected from a single female (Ćirković et al, 2002; FAO, 2006). The aim of this study was to investigate the possibility and efficiency of out off season artificial spawning of KOI

carp, the impact of body mass of females on the number of eggs, as well as the correlation between the percentage of migrated oocysts and spawning success.

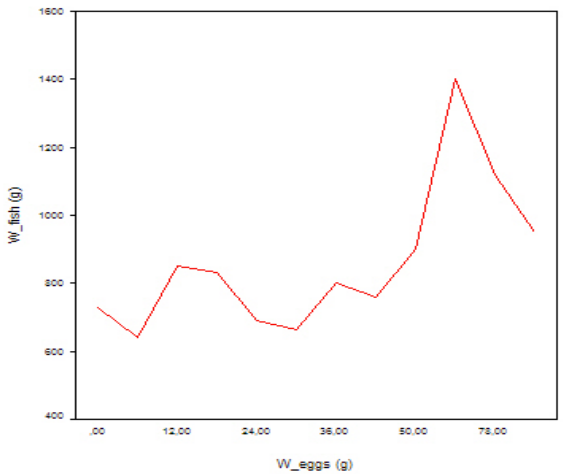
MATERIALS AND METHODS

Spawning was done at Gan Shumel Fisher Hatchery and Breeding Centre in Israel in December. Thirty females KOI-carp (*Cyprinus carpio L.*) were placed in 10 plastic tanks, cca 450 liter. Carp males were kept in separated ponds of carp female brood fish, in order to avoid uncontrolled reproduction. Brood fish were kept in water saturated with oxygen, within the temperature range of 20-24 ºC. The tanks were covered with a net, to prevent the fish from jumping out of the tank. From each tank, one female was biopsied. Females were anesthetized by 99% 2 phenoxyethanol and the biopsy of ovary was done via genital opening by inserting a 3 mm plastic catheter into the gonad. Carp eggs are opaque and the only way to examine the position of GV (germinal vesicle) under the binocular microscope is to make them transparent. The ovarian sample (of about 100 oocytes) was cleared in SERA solution (ethanol 60%, formalin 30% and acetic-acid 10%). Within 3 min the oocytes became translucent and remained so for an additional 5 min. The position of the germinal vesicle and ripeness of female was determined and the number of oocytes at each stage was recorded. Total weight of all females was calculated and it was around 40 kg. Hormones for injection were prepared for all fish together. 10 ml of 0,9 %NaCl saline was added to marked vial with "10 kg of CPE" (CPE-carp pituitary extract) and was mixed by hand. Final concentration was 1 kg/ml for each kg of fish that was injected with 1 ml of solution. The dose of hormones was divided in two doses. After the anesthesia of the fish, around 11 00h, intramuscular injection of cCPE hormone was given in the base of the dorsal fin - priming injection. Around 24 00 h fish received a second - inducting injection. The area of injecting was gently massaged followed with the withdrawal of the needle after injection to aid distribution of the extract into musculature and prevention of its backflow. 10 males were injected with 70% of the dose CPE that used for females. The day after, the spawning was occurred little bit later than we expected. Eggs were gently squeezed in the dry bowl and letter a small volume of sperm was added and mixed carefully together „the dry method". Physiological solution-saline was used to prolong the fertilization and rinsing solution (dilution of milk) to remove the stickiness of common carp eggs was added afterwards. Incubation was carried out in Zoug jars. For statistical analyses of correlations were used SPSS for Windows and Excel (MS Office).

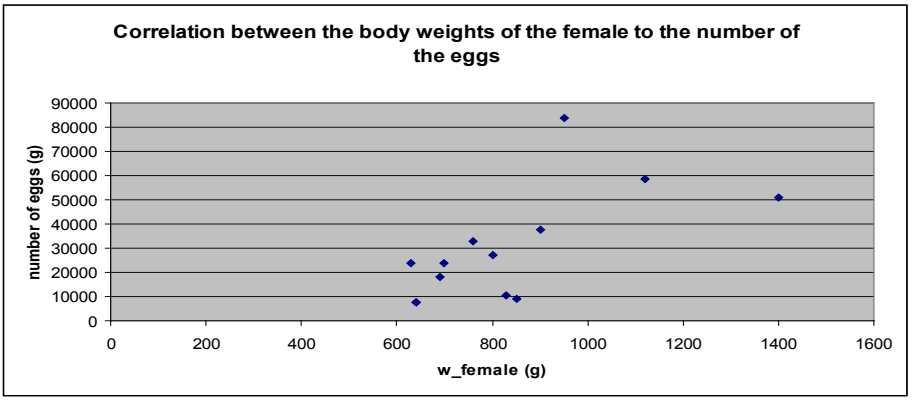
RESULTS AND DISCUSION

With total number of fishes n = 30 and number of spawned females n = 13 we had 43.33 % of spawning success. We measured the Spearman's correlating coefficient between body weight of fish and weight of eggs (Graph 1), correlation between body weight of fish and number of the eggs (Graph 2) and the correlation between the % of migrating oocytes to spawning success (Graph 3). In correlation between the percent of migrating oocytes to spawning success, only eight of the females with biopsy spawned (Table 1). There is significant correlation (F=0.709) between the body weight of the female to the weight of eggs and a significant correlation (F=0.642) between the body weight of the female to the number of the eggs. We found negative correlation (F = -0.530) between the percentage of migrating oocytes to spawning success which con-

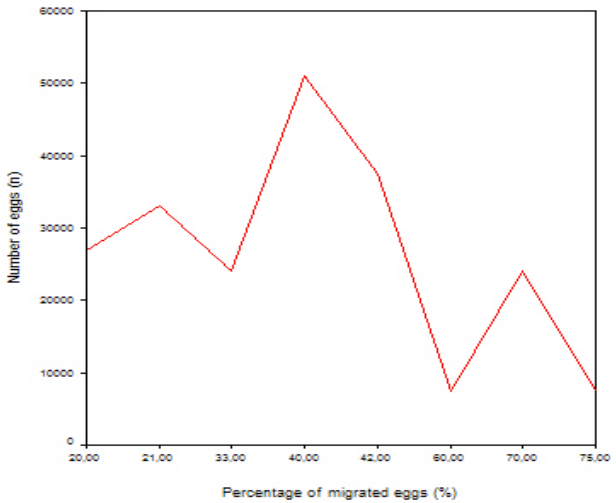
tradict with many spawning researches. We assume that the reason can be related due to environmental circumstances, as well as the quality of spawners.



Graph 1. Correlation between the body weight of the female to the weight of eggs (SPSS for Windows); F = 0,709



Graph 2. Correlation between the body weights of the females to the number of the eggs F = 0,642657



Graph3. Correlation between the % of migrating oocytes to spawning success; F=-0.530

Table 1. Percentage of migrated oocytes and number of eggs

N of females	% of migrated oocytes	N of eggs
1	70	24000
2	60	7500
3	40	51000
4	75	7500
5	20	27000
6	21	33000
7	33	24000
8	42	37500

To induce and synchronize ovulation and spermiation by hormonal stimulants, fish receive injection of pituitary gland, calibrated pituitary extract or a synthetic GnRH (Drori et all., 1994). It is recommended to use cCPE at the beginning and end of the spawning season when the LH content in the pituitary is low, and synthetic GnRH in mid-season and in field spawning. (Yaron et all., 2009). In our trial of induced spawning of carp, we used cCPE because of winter time and out off-season of spawning. Concerning hatching performance, hatching rate of 43.33% was slightly lower compared to 95% reported by Horvath and Lukowicz (1982) and 90-100% reported by Billard et all. (1995). Latency time is also highly dependable on water temperature. Latency time of our treated carps was little postponed due to small system heating error. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a very big success because it was done out off-season. Carp pituitary extract (CPE) have been used in most hatcheries, however the increased production targets and the cost of this biological material led to consider alternative approaches (Yaron 1995).

CONCLUSIONS

Concerning hatching performance, hatching rate of 43,33 % was slightly lower compared to results reported by others. This could be attributed to immature of some individuals, since the experiment was performed before the reproductive period, in order to secure the spawning response in fully mature fish. The recognition of the best moment for applying hormonal induction in cyprinid artificial spawning is very important. Before any action on fish, it must be anesthetized and handling must be done very gently. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a very big success because it was done out off-season.

ACKNOWLEDGMENTS

The authors wish to thank The Hebrew University of Jerusalem, The Robert H. Smith, Faculty of Agricultural, Food & Environmental Quality Sciences – Division for External Studies.

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